## THE ANALYSIS OF SUSPECTED HYBRIDS, AS ILLUSTRATED BY BERBERIS X GLADWYNENSIS

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During the last fifteen years a considerable portion of my time has been spent in attempting to measure the importance of hybridization in the evolution of natural populations. More than a score of genera have been studied intensively in the wild (and whenever feasible in the breeding plot as well) with students and associates, though as yet only a fraction of these studies has been published. Various techniques have been worked out for dealing with such problems (Anderson, 1949, chap. 6), and as their validity has been more widely recognized (Stebbins, 1952) they are being more widely adopted. In the course of helping workers elsewhere to adapt these methods to their own problems I have become increasingly aware that in part they are based upon a scrutiny of the original material more exhaustive than is customary in many laboratories. It has accordingly seemed expedient to describe the procedure in more elementary detail than hitherto. Instead of discussing the complexities of hybridization in natural populations I have chosen the relatively simple example of an apparent hybrid which arose spontaneously in the garden of Mrs. J. Norman Henry, of Gladwyne, Pennsylvania. This seems a particularly happy choice since it also serves to demonstrate the usefulness of these methods in dealing with horticultural material of unknown ancestry.

A vigorous young barberry was found coming up in the shelter of a large bush of Berberis verruculosa in Mrs. Henry's garden. Berberis verruculosa is a dense, evergreen-leaved plant, certainly the most distinctive of the hardy barberries. The seedling, so obviously related that it must have been a seedling of B. verruculosa, was nevertheless so different from it that Mrs. Henry had supposed it was probably a spontaneous hybrid. It was different from any barberry known to her or to me and it exhibited the hybrid vigor which is the mark of so many hybrids. Furthermore, many garden hybrids of Berberis are known and Mrs. Henry at various times had grown a number of other species of Berberis in her garden. With her permission a precise examination was made both of the putative hybrid and of Berberis verruculosa to establish the probable ancestry.

The following is the procedure established in this laboratory for such examinations:

1. Choose comparable material of parent and hybrid.

2. Examine, describe, and measure each feature item by item. Use great care to work with truly comparable material. Do not attempt comparisons of a branch grown in the shade from one specimen and one grown in the sun from the other. If one is from a fruiting branch, then the other should be also. Use great care in distinguishing between long shoots and short shoots. Many plants without such conspicuously heterogeneous shoot systems as the Ginkgo have a more or less well-defined short shoot-long shoot system which requires careful examination to perceive.

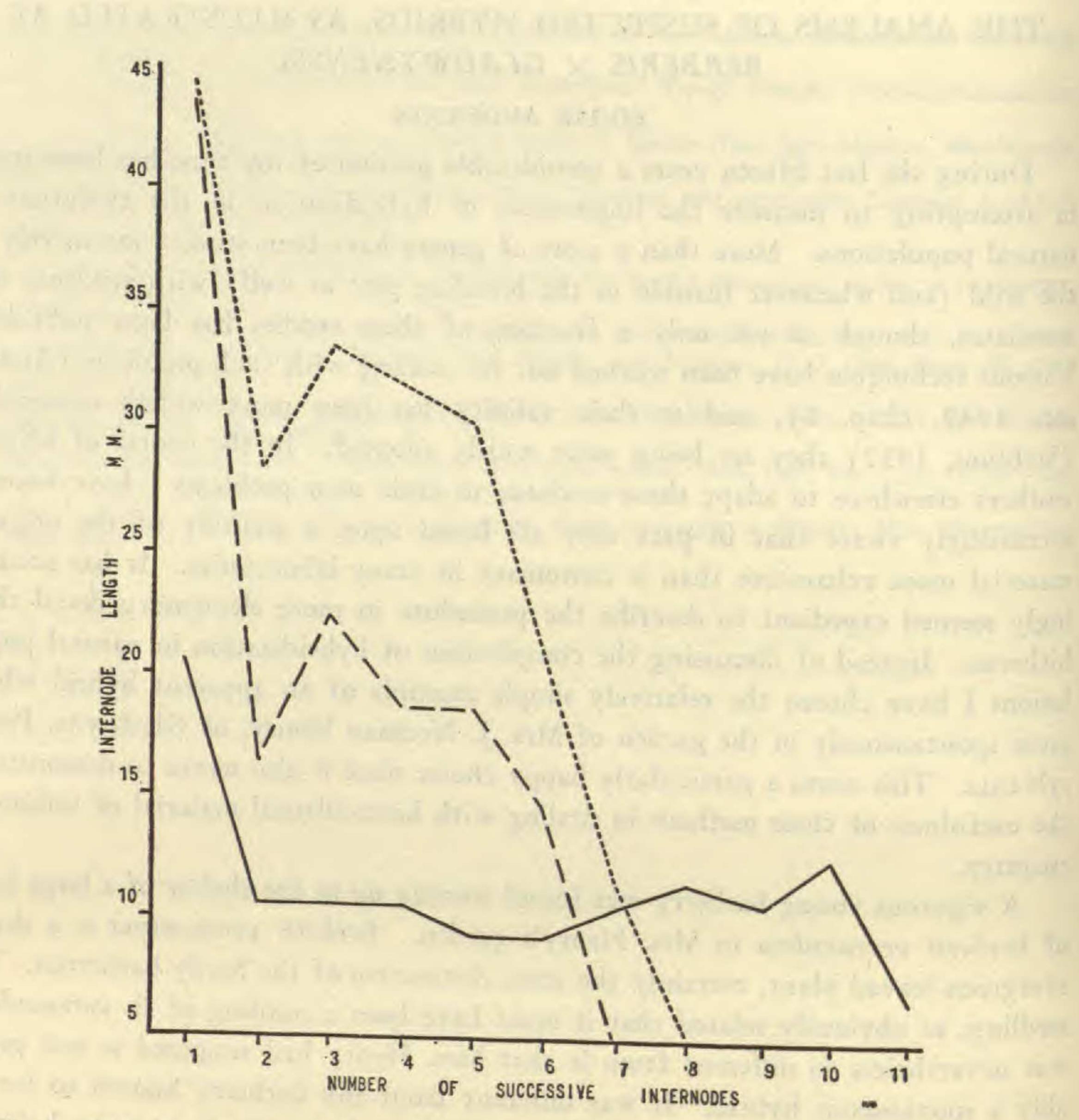


Fig. 1. Internode diagrams of comparable branchlets of two species of barberry and their putative hybrid. Solid line, Berberis verruculosa; long dashes, Berberis X gladwynensis; short dashes, Berberis Julianae. As explained in Anderson and Schregardus (1944), these diagrams are these points with an imaginary line. The first internodes from the base to the tip and connecting for instance, is 45 mm. long, the second is 28 mm., the third, 33 mm., etc.

3. (And, most important): Conduct the entire examination, if possible, against a plain neutral background. Cover the table top with clean wrapping paper or some such material. Remove all distracting objects such as pencils, paper clips, erasers, etc., out of the immediate field of vision. The eye can work much more efficiently in the perception of resemblances and differences if it can concentrate upon the problem in hand. Work in a good light and have a good dissecting microscope. Choose a series of comparable branches and lay them out out side by side with equal spacing. Choose a series of comparable leaves and lay them direction. These seem like points much too elementary for serious scientific dis-

cussion, and yet in assisting other people to use my methods I nearly always find that these precautions have not been followed and that it is difficult to convince other scholars of their supreme importance. This is one of those ridiculously simple matters which are far more important than they seem and which are indicative of true scientific precision. Specific differences are frequently subtle and they can be apprehended much more readily if one uses comparable material, a neutral background, and a good light.

- 4. Make measurements with good steel calipers and a steel metric rule.
- 5. Pay particular attention to pubescence, pubescence pattern, internode pattern (Anderson and Schregardus, 1944), branching pattern. Careful analysis will show that nearly all closely related species of the higher plants differ significantly (and with enough study, definably) in their internode patterns.

Comparable branches were accordingly chosen from Berberis verruculosa and the seedling. Both were selected from well-developed branches of flowering age, growing in almost full sun and borne upon the upper parts of their respective plants. After these two branches had been examined carefully against a neutral background, comparable branchlets were chosen for further study as follows: Proceeding from the tip, the first strong secondary branch (all of the current season's growth) was selected for study. The internode lengths of this branchlet were measured to the nearest millimeter, the results being plotted as an internode diagram (fig. 1).

Hybrids are usually intermediate between their two parents when due allowances are made for heterosis, for growth-pattern differences, and for possible differences in ploidy. From a study of fig. 1, it is possible to make several predictions as to the putative male parent of the seedling barberry. B. verruculosa is shown to have more and shorter internodes than the seedling. Its basal internode is only about twice as long as the other internodes and the remaining internodes are subequal. The seedling has a basal internode more than twice as long as the others and the remaining internodes are not at all uniform in length and seem to have a definite pattern of decreasing length toward the tip. We therefore predict that the putative parent (when comparable material is studied) should have conspicuously non-uniform internode lengths with a very long internode at the base of such branches and decreasingly short ones as the tip is approached, probably in a well-defined pattern.

Leaf comparisons were then made. Choosing comparable leaves proved to be difficult but not impossible. Leaf size and shape were uniform in B. verruculosa but variable in the seedling. Care had to be taken, therefore, to choose exactly comparable material. Most of the primary leaves in these barberries are spines. The leafy leaves are borne on short secondary branches in the axils of the spine-leaves, giving the appearance of little clusters up and down the branches. In B. verruculosa all the leaves on the plant were superficially similar in size, shape, and serration. In the seedling they differed markedly. Those in clusters arising from the older wood were variable. Among them were leaves which were narrower

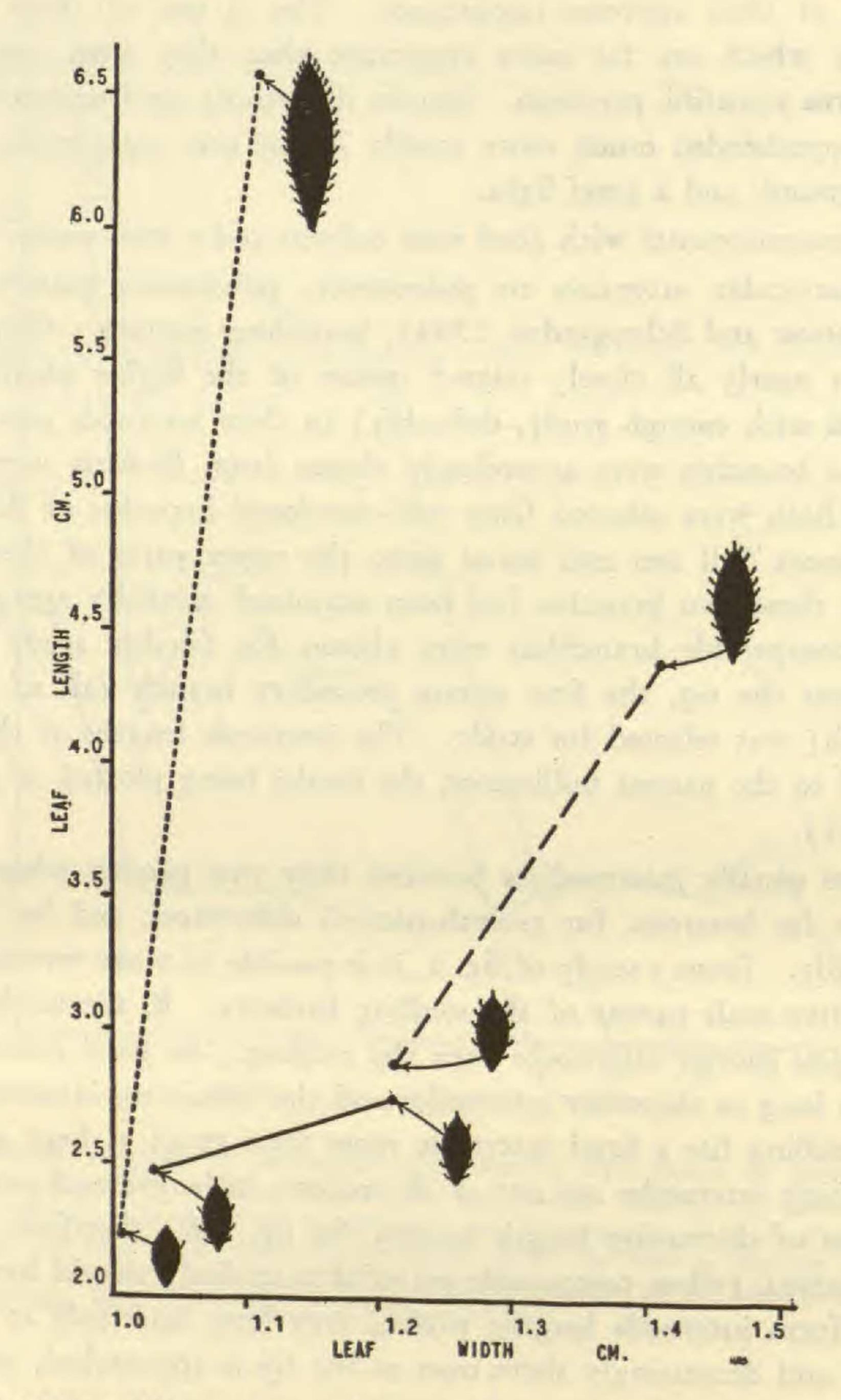


Fig. 2. Relationships of size, shape, and number of marginal setae in leaves from young and mature spurs for two species of barberry and their putative hybrid. As in the previous figure, a solid line indicates B. verruculosa, short dashes, B. Julianae, and long dashes, Berberis × gladwynensis, their putative hybrid. The figure shows simultaneously the length, width, and seta number of comparable leaves from young and from old spurs; the slope of the lines also indicates the change from young to old: Berberis Julianae changes conspicuously in size, shape and seta number from young to old spurs; B. verruculosa scarcely at all; their hybrid is intermediate in degree of matic, are all to the same scale and represent average values from actual measurements.

in shape, larger in size and with more strongly developed setae at the tips of the serrations along their edges. Careful examination then showed that B. verruculosa had a very slight tendency in the same direction though not strong enough to be readily apparent without examination. Measurements showed that while Berberis verruculosa and the seedling had about the same number of setae per leaf on the new wood, the seedling had nearly twice as many as verruculosa on the old wood. Figure 2 shows all these relationships. The putative parent for which we are searching should have leaves on its new wood as small as those of verruculosa, or even smaller, with greatly differentiated leaves on the older wood. Some of the leaves on the older wood should be very long, proportionately narrow, and with many setae along their edges.

As, in this fashion, one proceeds feature by feature in the careful comparison of the one parent with the hybrid, he becomes progressively better acquainted with the general ground plan of the plant and is increasingly capable of making precise comparisons and valid inferences. Before long it is possible to draw up precise descriptions not only of B. verruculosa and its hybrid seedling but also of the putative male parent. Such a comparison is presented below in tabular form, including the predictions as to the male parent.

verruculosa	gladwynensis	unknown (Julianae)
Branches arching, sub-horizontal	Branches sub-arching, some nearly erect	Branches straight, erect to horizontal
Internodes short, uniform	Internodes medium, variable	Internodes long, very variable
Branchlets densely glandular	Branchlets at most sub-glandular	Branchlets eglandular
New growth scarcely differentiated	New growth clearly differentiated	New growth strongly differentiated
Mature bark barely ribbed	Mature bark definitely ribbed	Mature bark strongly ribbed and furrowed
Wood greenish	Wood yellowish-green	Wood bright yellow-green
Spines up to 1.2 cm.	Spines up to 1.3 cm.	Spines up to 1.5 cm.
Leaves glossy, dark green above, glaucous below	Leaves somewhat glossy, medium-dark above, sub-glaucous below	Leaves not glossy, bright green above, light green below
Fall color a more or less general purple	Fall color stronger on newest growth, occasional colored leaves	Brilliant fall color
Leaves on new and old growth scarcely	Leaves on new and old growth definitely	Leaves on new and old growth strongly differentiated
Largest leaves on old spurs 27 mm. × 12 mm.	Largest leaves on old spurs 43 mm. X 14 mm.	Largest leaves on old spurs 65 mm. X 11 mm.
10 marginal setae at most	20 marginal setae at most	Over 30 marginal setae
Flowers borne singly or in pairs	Flowers in fascicles of 5 to 8	Flowers in fascicles of 15 to 25
Stigma sessile	Stigma sub-sessile	Stigma definitely not sessile style of at least 1 mm.

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As the description began to resolve itself my knowledge of barberries was sufcient to suggest that Berberis Julianae was probably the barberry we were looking for. As soon as I made this suggestion Mrs. Henry informed me that a large bush of this species had once stood just a few feet away from B. verruculosa but it had been damaged in a windstorm and removed. Many of the technical points in the description, however, involved matters which were completely outside my knowledge of barberries. Predictions as to the numbers of setae on the mature leaves, the number of flowers per fascicle, the presence of a style, etc., were drawn up with no knowledge of what they might be like in B. Julianae. The entire hypothetical description was then run down in Rehder's key (Rehder, 1940) to the barberries just as if one had the specimen actually in his hand. It led to B. Julianae and fitted the description of that species precisely, down to the most technical detail.

There can be little doubt, therefore, that the seedling was a spontaneous hybrid, B. verruculosa × B. Julianae. I am accordingly describing it as:

BERBERIS X gladwynensis hyb. nov. Intermediate between its parents B. verruculosa and B. Julianae. Flowers in fascicles of 5 to 8, stigma subsessile, leaves subglaucous below, nearly evergreen. Type: Henry, in the herbarium of the Philadelphia Academy of Natural Sciences; from the type bush, E. Anderson s. n., MBG.

It is a pleasure to name this handsome barberry after the site of Mrs. Henry's garden which has long been a mecca to botanists and gardeners alike.

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<sup>&</sup>lt;sup>1</sup>Berberis gladwynensis hyb. nov. inter parentes B. verruculosam et B. Julianam. Flores in fasciculis 5-8, stigmate subsessile. Folia fere semperviridia, subter subglauca.